METHOD FOR OBTAINING NATURAL PRODUCTS FROM PLANT MATERIAL

Background of the Invention

Birch bark is a low-value waste product in the forest industry today. Ekman, R., Holzforschung, (1983) 37, 205. Approximately 230,000 tons of birch bark are generated per year. For example, a single paper mill can generate 70 tons of birch bark per day. Thus, vast quantities of birch bark and its chemical components are available.

Birch bark is a potential source of a variety of organic chemicals. Several triterpenoids have been identified in birch bark extracts. For example, lupeol, betulin, betulinic aldehyde, betulinic acid, methyl betulinate, lupenone, betulonic aldehyde, betulonic acid, β-amyrin, erythrodiol, oleanolic aldehyde, oleanolic acid, methyl leanolate and acetyl oleanolic acid are all present in the outer bark of *Betula verrucosa*. Eckerman, C., (1985) Paperi ja Puu, No. 3, 100. In addition, several suberinic acids isolated from birch bark, as well as several triterpenoids, have been identified in the bark of *Betula verrucosa*. Ekman, R., Holzforschung, (1983) 37, 205.

The chemical constituents of birch bark are useful in pharmaceutical and industrial applications. For example, U.S. Pat. No. 5,750,578 discloses that betulin possesses antiviral properties and is useful to treat herpes virus. Betulin also possesses anti-feedant activity against boll weevils, and anti-inflammatory activity (Miles, D. H., 1994, J. Agric. Food. Chem., 42, 1561-1562 and Recio, M., Planta Med., 1995, 61, 9-12. In addition, betulin showed cough suppressant and expectorant effects. Jinuhua, W., Zhongguo Yaoxue Zazhi, (1994), 29(5), 268-71. Betulin is also a useful starting material for preparing alobetulin and derivatives thereof, which posses useful pharmacological properties.

Betulin can be converted to betulinic acid, which is useful as a therapeutic agent. For example, Pisha, E. et al., (1995) J. M. Nature Medicine, 1, 1046-1051 discloses that betulinic acid has anti-tumor activity against human melanoma, e.g., MEL-1, MEL-2 and MEL-4. In addition, Fujioka, T. et al., J.

Nat. Prod., (1994) 57, 243-247 discloses that betulinic acid has anti-HIV activity in H9 lymphocytic cells.

Current methods for isolating the chemical constituents of birch bark are deficient in several ways. For example, betulin has been extracted from the bark of white-barked birches in amounts up to 20%, based on the dry weight of the bark. Elkman, R., (1983) Holzforsch, 37, 205; Ohara, S., et al., (1986) Mokuza Gakkaishi, 32, 266. In addition, betulin has been isolated from outer birch bark waste of *Betula verrucosa* by liquid extraction employing boiling organic solvents and subsequent recrystallization. Eckerman, C., (1985) Paperi ja Puu, No. 3, 100. While current processes afford acceptable yields of betulin (e.g., 11-20%), these processes suffer from several major drawbacks. Specifically, the current methods employed to isolate not only betulin, but other components in birch bark (e.g., lupeol and betulinic acid) are costly, inefficient an/or are unsafe.

Russian Patent Nos. RU2175326 (publication date 27 October 2001) and RU2192879 (publication date 20 November 2002), discloses methods of preparing betulin, and derivatives thereof. The methods disclosed in Russian Patent No. RU2192879 include birch bark milling, separation of birch bark fibers, extraction of birch bark, separation of solution from extracted birch bark, and solvent removal from solution. The birch bark extraction is carried out with toluene at temperature of 90°C-110°C for 1.5-3.0 h, and the solution is filtered at a temperature of 40°C-50°C. The toluene betulin solution is cooled for 6-10 h to a temperature of 15°C - 5°C.

Published U.S. Patent Application US 2003/0153776 A1 ("the '776 patent application), assigned to Boehringer Ingelheim Pharma, discloses a process for obtaining betulin from birch bark (see Abstract). The process includes extracting birch bark with a high-boiling, water-immiscible solvent, and extracting this extract with a dilute aqueous base (see claim 1). The methods in the '776 patent application are disclosed to provide betulin. No other triterpenoids (e.g., lupeol, betulinic acid, or a combination thereof) are obtained with the methods described therein. Additionally, only 4 wt.% of betulin is obtained with the methods described therein. The use of charcoal is also required in the methods described therein. It is believed that the use of a decolorization agent such as activated charcoal decreases the overall yield of

betulin. The methods described in the '776 patent application are not able to effectively remove triterpenoids such as betulin-3-caffeate, betulinic acid, lupeol, esters of fatty acids, fatty acids, polyphenols and tannins, from the birch bark or birch bark extract. The process described in the '776 patent application is unsuitable for the industrial scale recovery of betulin, as well as lupeol and betulinic acid. The yields and purities of betulin disclosed in the '776 patent application are believed to be erroneous, utilizing the processes described therein. Even if accurate, the yields and purities of betulin disclosed in the '776 patent application can be improved. The processes described therein cannot be practiced on larger, industrial scales (e.g., kilograms or tons).

A need therefore exists for safer, more cost-efficient and/or more efficient methods to obtain commercial quantities (e.g., tons) of betulin; as well as commercial quantities (e.g., kg) of lupeol and betulinic acid from birch bark.

Summary of the Invention

The process of the present invention is suitable for obtaining betulin, lupeol and betulinic acid from birch bark, in a yield of about 10 wt.% to about 12 wt.%, about 2.5 wt.% and about 2 wt.%, respectively. The betulin, lupeol and betulinic acid obtained using the methods of the present invention are relatively pure. For example, the betulin will typically include less than about 0.02 wt.% of betulinic acid. Additionally, employing methods of the present invention, commercial quantities (e.g., tons) of betulin; as well as commercial quantities (e.g., kg) of lupeol and betulinic acid can be obtained from birch bark.

The methods of the present invention can also be employed to effectively remove betulin-3-caffeate, betulinic acid, esters of fatty acids, fatty acids, polyphenols and tannins from the birch bark or birch bark extract. Specifically, betulin-3-caffeate and esters of fatty acids are effectively hydrolyzed upon contact and heating with the base. Upon hydrolysis, the betulin-3-caffeate is converted to betulin, which increases the overall yield of betulin; and the esters of fatty acids provide methanol and a salt of betulinic acid, which precipitates and can be removed from the reaction mixture. The betulinic acid precipitates as a basic salt, and can be removed from the reaction mixture. The polyphenols also precipitate as polyphenolates, which can be removed from the reaction mixture.

The present invention provides a method for obtaining a natural product from plant material. The method includes: (a) contacting plant material with a solvent to provide a first mixture; (b) separating the plant material from the solvent to provide a first extract; (c) contacting the first extract with an aqueous base to provide a second mixture; (d) heating the second mixture in an organic solvent that (i) is water-immiscible, (ii) is capable of forming an azeotropic mixture with water, or (iii) has a boiling point of at least 100°C (e.g., at least about 120°C, at least about 140°C, or at least about 160°C), effective to distill off water present in the second mixture, thereby providing a third mixture; (e) separating solids from the third mixture to provide a fourth mixture; and (f) concentrating the fourth mixture, or precipitating solids from the fourth mixture, to provide a natural product.

The present invention also provides a method for selectively obtaining one or more non-acidic compounds from plant tissue in the presence of one or more acidic compounds. The method includes: (a) contacting plant material with a solvent to provide a first mixture; (b) separating the plant material from the solvent to provide a first extract; (c) contacting the first extract with an aqueous base to provide a second mixture; (d) heating the second mixture in an organic solvent that (i) is water-immiscible, (ii) is capable of forming an azeotropic mixture with water, or (iii) has a boiling point of at least 100°C (e.g., at least about 120°C, at least about 140°C, or at least about 160°C), effective to distill off water present in the second mixture, thereby providing a third mixture; (e) separating solids from the third mixture to provide a fourth mixture; and (f) concentrating the fourth mixture, or precipitating solids from the fourth mixture, to provide a natural product.

The present invention also provides a method for obtaining betulin from birch bark. The method includes: (a) contacting birch bark with a solvent to provide a first mixture; (b) separating the birch bark from the solvent to provide a first extract; (c) contacting the first extract with an aqueous base to provide a second mixture; (d) heating the second mixture in an organic solvent that (i) is water-immiscible, (ii) is capable of forming an azeotropic mixture with water, or (iii) has a boiling point of at least 100°C (e.g., at least about 120°C, at least about 140°C, or at least about 160°C), effective to distill off water present in the second

mixture, thereby providing a third mixture; (e) separating solids from the third mixture to provide a fourth mixture; (f) contacting the fourth mixture with binder to provide a fifth mixture; (g) filtering the fifth mixture to provide a solution; (h) concentrating the solution to provide betulin, or precipitating betulin from the solution.

The present invention also provides a method for obtaining lupeol from birch bark. The method includes: (a) contacting birch bark with a solvent to provide a first mixture; (b) separating the birch bark from the solvent to provide a first extract; (c) contacting the first extract with an aqueous base to provide a second mixture; (d) heating the second mixture in an organic solvent that (i) is water-immiscible, (ii) is capable of forming an azeotropic mixture with water, or (iii) has a boiling point of at least 100°C (e.g., at least about 120°C, at least about 140°C, or at least about 160°C), effective to distill off water present in the second mixture, thereby providing a third mixture; (e) separating solids from the third mixture to provide a fourth mixture; (f) precipitating solids from the fourth mixture; (g) separating the solids from the fourth mixture to provide a mother liquor; (h) concentrating the mother liquor to provide a fifth mixture; (i) washing the fifth mixture with a polar organic solvent to provide a sixth mixture; (j) crystallizing the sixth mixture with a non-polar organic solvent to provide a solution; (k) concentrating the solution to provide crude lupeol. The process can optionally further include (l) recrystallizing the lupeol from a polar organic solvent to provide pure lupeol and (m) drying the pure lupeol.

The present invention also provides a method for obtaining betulinic acid from birch bark. The method includes: (a) contacting birch bark with a solvent to provide a first mixture; (b) separating the birch bark from the solvent to provide a first extract; (c) contacting the first extract with an aqueous base to provide a second mixture; (d) heating the second mixture in an organic solvent that (i) is water-immiscible, (ii) is capable of forming an azeotropic mixture with water, or (iii) has a boiling point of at least 100°C (e.g., at least about 120°C, at least about 140°C, or at least about 160°C), effective to distill off water present in the second mixture, thereby providing a third mixture; (e) separating solids from the third mixture; (f) washing the solid with water to provide a second solids; (g) neutralizing or acidifying the second solids with an aqueous acid,

thereby providing a fourth mixture; (h) separating crude betulinic acid from the fourth mixture. The process can optionally further include (i) crystallizing the crude betulinic acid from polar organic solvent to provide pure betulinic acid and (j) drying the pure betulinic acid.

The present invention also provides a method for purifying an extract of a natural product. The method includes: (i) contacting an extract of a natural product with an aqueous base, to provide a first mixture; (ii) heating the first mixture in an organic solvent that (i) is water-immiscible, (ii) is capable of forming an azeotropic mixture with water, or (iii) has a boiling point of at least 100°C (e.g., at least about 120°C, at least about 140°C, or at least about 160°C), effective to distill off water present in the first mixture, thereby providing a second mixture; (iii) separating solids from the second mixture, to provide a third mixture; and (iv) concentrating the third mixture or precipitating solids from the third mixture, to provide a purified natural product.

The contacting of the extract with an aqueous base and heating in an organic solvent that (i) is water-immiscible, (ii) is capable of forming an azeotropic mixture with water, or (iii) has a boiling point of at least 100°C (e.g., at least about 120°C, at least about 140°C, or at least about 160°C), effective to distill off water present, can specifically include azeotropic distillation.

The present invention also provides a compound obtained from the method of the present invention.

The present invention also provides a pharmaceutical composition that includes a pharmaceutically acceptable carrier and a compound obtained from the method of the present invention.

The present invention also provides a cosmetic composition that includes a cosmetically acceptable carrier and a compound obtained from the method of the present invention.

Brief Description of the Figures

Embodiments of the invention may be best understood by referring to the following description and accompanying drawings which illustrate such embodiments. The numbering scheme for the Figures included herein are such that the leading number for a given reference number in a Figure is associated with the number of the Figure. Reference numbers are the same for those

elements that are the same across different Figures. For example, a block flow diagram depicting outer birch bark (2) can be located in Figure 1. However, reference numbers are the same for those elements that are the same across different Figures. In the drawings:

Figure 1 illustrates a block flow diagram depicting the isolation of betulin, lupeopl, and/or betulinic acid from birch bark, employing distillation.

Figure 2 illustrates a block flow diagram depicting the isolation of acidic components (natural products) and non-acidic components (natural products) from plant material, employing distillation.

Detailed Description of the Invention

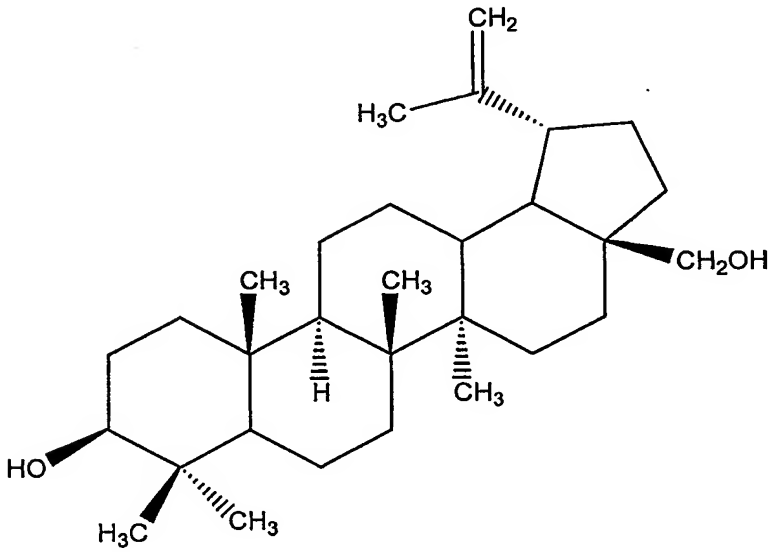
Specific values listed below for ranges are for illustration only; they do not exclude other defined values or other values within defined ranges.

Definitions

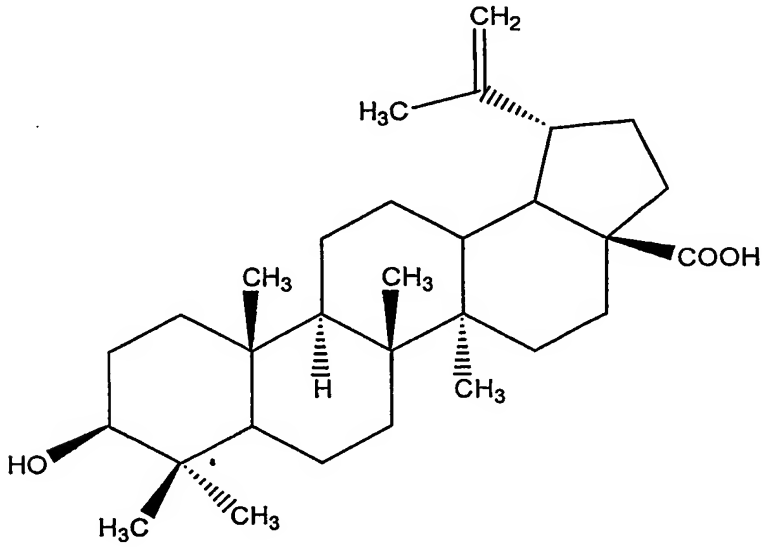
As used herein, "triterpene" or "triterpenoid" refers to a plant secondary metabolite that includes a hydrocarbon, or its oxygenated analog, that is derived from squalene by a sequence of straightforward cyclizations, functionalizations, and sometimes rearrangement. Triterpenes or analogues thereof can be prepared by methods known in the art, i.e., using conventional synthetic techniques or by isolation from plants. Suitable exemplary triterpenes and the biological synthesis of the same are disclosed, e.g., in R.B. Herbert, The Biosynthesis of Secondary Plant Metabolites, 2nd. Ed. (London: Chapman 1989). The term "triterpene" refers to one of a class of compounds having approximately 30 carbon atoms and synthesized from six isoprene units in plants and other organisms. Triterpenes consist of carbon, hydrogen, and optionally oxygen. Most triterpenes are secondary metabolites in plants. Most, but not all, triterpenes are pentacyclic. Examples of triterpenes include betulin, allobetulin, lupeol, friedelin, and all sterols, including lanosterol, stigmasterol, cholesterol, β-sitosterol, and ergosterol.

As used herein, "betulin" refers to 3β,28-dihydroxy-lup-20(29)-ene. Betulin is a pentacyclic triterpenoid derived from the outer bark of paper birch trees (*Betula papyrifera, B. pendula, B. verucosa*, etc.). It can be present at

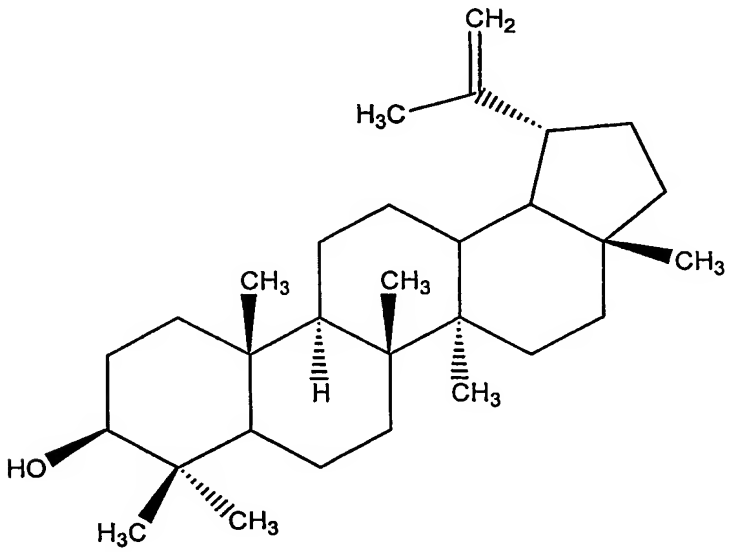
concentrations of up to about 24% of the bark of white birch. Merck Index, twelfth edition, page 1236 (1996). Structurally, betulin is shown below:



As used herein, "betulinic acid" refers to 9-hydroxy-1-isopropenyl-5a,5b,8,8,11a-pentamethyl-eicosahydro-cyclopenta[a]chrysene-3a-carboxylic acid. Structurally, betulinic acid is shown below:



As used herein, "lupeol" refers to lup-20 (29)-en-3 β-ol. Lupeol is also found in birch bark and in other plant sources. Lupeol is present at concentrations of about 1.5-3% of the birch bark and at up to about 8.2% in Canavalia ensiformis, a plant widespread in the humid tropics of Asia and Africa. Structurally, lupeol is shown below:



As used herein, "natural product" refers to naturally occurring compounds that are end products of secondary metabolism; often, they are unique compounds for particular plants or species of plants. In a specific embodiment of the present invention, the compounds can be derived from plant material, e.g., birch bark. Such compounds can include, e.g., triterpenes or triterpenoids. Additionally, the natural product can include a single compound, or can include one or more different compounds. Additionally, these one or more different compounds can be structurally related or structurally unrelated.

As used herein, a "non-acidic compound" refers to a natural product that comprises lupeol, betulin, taxol, paclitaxel, echinacea extract, valerian root extract, ginkgolide A, ginkgolide B, ginkgolide C, bilobalide, garlic extract, ginseng extract, aloe gel, barbaloin, cranberry extract, eleutheroside A, eleutheroside B, eleutheroside C, eleutheroside D, eleutheroside E, eleutheroside G, kava extract, dill seed oil, kola extract, quinoline alkoloids, or a combination thereof.

As used herein, an "acidic compound" refers to a natural product that acidic compounds comprises betulin acid, betulin-3-caffeate, tannin, lipid, phenol, caffeic acid, cichoric acid, valerenic acid, isovaleric acid, flavonoid, quercetin, kaempferol, catechin, lignin, shikimic acid, succinic acid, amino acid, nicotinic acid, pantothenic acid, anthraquinone, acidic galactan, benzoic acid, quinic acid, malic acid, citric acid, hippuric acid, phenolic acid, ferulic acid, . chlorogenic acid, norsolorinic acid, or a combination thereof.

As used herein, "plant material" or "plant tissue" refers to a collection of similar cells of a plant, that typically act together to perform a particular function. The term refers to the tissue of any organism of the plant kingdom, as opposed to one of the animal kingdom or of the kingdoms of Fungi, Protista, or Monera. The plant tissue can be any portion or portions of the plant (e.g., bark, roots, leaves, flowers, needles, bulbs, berries, rhizomes, rootstocks, stems, and seeds), as well as the entire plant. The tissues of a plant ("plant tissue") generally fall into three main categories: dermal tissue, ground tissue, and vascular tissue. Dermal tissue refers to the "skin" layer of all plant organs and is responsible for environmental interaction (light passage, gas exchange, pathogen recognition and protection, color display, etc.). Dermal tissue is composed of epidermal cells, closely packed cells that secrete a waxy cuticle that aids in the prevention of water loss. Ground tissue lies between dermal tissue and vascular tissue. The ground tissue comprises the bulk of the primary plant body. Parenchyma, collenchyma, and sclerenchyma cells are common in the ground tissue. In roots, the ground tissue may store sugars or starches to fuel the spring sap flow; in leaves, the ground tissue is the layer responsible for photosynthesis (the mesophyll). Vascular tissue transports food, water, hormones and minerals within the plant. Vascular tissue includes xylem, phloem, parenchyma, and cambium cells.

As used herein, "bark" refers to the dry, dead outer covering of woody branches, stems and roots of plants that is very distinct and separable from the wood itself. It includes all tissue outside the cambium (growth layer between bark and wood).

As used here the terms "leaf" or "leaves" refer to those parts of a plant which grow along the sides of branches or stems or at the bases of plants. Most

are green and contain chlorophyll, though they vary in their shapes and sizes. Leaves are the part of the plant that ordinarily performs photosynthesis (the process that converts sunlight and carbon dioxide into energy).

As used herein, "needle" generally refers to a narrow stiff leaf, such as those of conifers (e.g., pine trees).

As used herein, "root" refers to the part of a plant, normally underground, that absorbs nutrients and anchors the plant into the ground.

As used herein, "bulb" refers to a spheroidal body growing from a plant either above or below the ground (usually below), which is usually a bud, consisting of a cluster of partially developed leaves, and producing, as it grows, a stem above, and roots below, (e.g., the onion or tulip bulb). A true bulb is a complete package containing next year's plant (flower) already forming inside. The contents of the bulb are often enclosed in protective, fleshy scales, which are held together by a small basal plate. The scales are modified leaves that contain enough nutrients to sustain the plant through dormancy and early growth. They may be loose and open like those of a lily, or tightly closed like those of a hyacinth. In many bulbs, a paper-thin tunic protects the scales (lilies don't have a tunic). Roots will grow from the bulb's basal plate.

As used herein, "berry" refers to any small fruit that is pulpy or succulent throughout, having seeds loosely imbedded in the pulp, such as the currant, grape, or blueberry. Berry can be further defined as an indehiscent fruit derived from a single ovary and having the whole wall fleshy, such as the grape or tomato. Furthermore, berries come in various structures including simple, such grape; blueberry, cranberry, or aggregate, such as blackberry; raspberry, strawberry mulberry.

As used herein, "rhizome" refers to a horizontal, usually underground stem that often sends out roots and shoots from its nodes (also called rootstalk or rootstock).

As used herein, "rootstock" refers to a robust plant that provides the root system in grafting, also known as a stock. Scions and buds are grafted and budded to a rootstock or stock. Rootstock also refers to the elongated and often thick rhizomes of certain perennial herbaceous plants such as the Iris, Aspidistra and Solomon's Seal.

As used herein, "stem" refers to the main (usually aerial) axis (sometimes referred to as the trunk or stalk) of a tree, shrub, or plant. "Stem" also refers to the part of the plant that supports the leaves, flowers or fruits of a plant, such as the peduncle of a fruit or the pedicel of a flower.

As used herein, "seed" refers to a ripened ovule, consisting of an embryo with one or more integuments, or coverings, such as an apple seed, a currant seed, dill seed, or kola nut seed. By germination, most seeds produces a new plant. "Seed" also refers to any small seedlike fruit, though it may consist of a pericarp, or even a calyx, as well as the seed proper, such as a parsnip seed or thistle seed. The seed proper has an outer and an inner coat, and within these the kernel or nucleus. The kernel is either the embryo alone, or the embryo enclosed in the albumen, which is the material for the nourishment of the developing embryo. The scar on a seed, left where the stem parted from it, is called the hilum, and the closed orifice of the ovule, the micropyle.

Plant

The plant can be a bryophyte or vascular plant. More specifically, the plant can be grass, flower or a tree and the plant tissue can be any part of the grass, flower or tree. Specific plants, flowers, and trees include, e.g., Moss (e.g., Club Moss), Horsetail, Fern, Conifer, Cycad, *Ginkgo biloba* (Ginkgo), *Taxus yunnanesis* (yew tree), *Echinacea spp.*, *Valeriana officinalis*, *Allium sativum* (garlic), *Panax ginseng*, aloe vera, *Vaccinium macrocarpon*, *Eleutherococcus senticosus*, *Piper methysticum*, dill, kola nut, and cinchona.

Another specific plant is the birch tree, wherein the suitable plant tissue can be the bark of the birch tree. As used herein, "birch" or "birch tree" refers to any of the several deciduous tress of the genus *Betula*. The birches comprise the family Betulaceae in the order Fagales. Birch trees include, for example, white birch, *B. alba*; sweet, black or cherry birch, *B. lenta*; monarch birch, *B. maximowicziana*; dwarf or arctic birch; *B. nana*; Japanese white birch, *B. platphyla japonica*; smooth-bark birch, *B. pubescens*; yellow birch, *B. alleghaniensis*; paper, white or canoe birch, *B. papyrifera*; gray birch, *B. populifolia*; river birch, *B. nigra*; and the European birches, *B. pubescens*; *B. alba* and *B. pendula*. Specifically, birch can be *B. alba*, *B. lenta*, *B.*

maximowicziana, *B. nana*, *B. platyphyla japonica*, *B. pubescens*, *B. alleghaniensis*, *B. papyrifera*, *B. populifolia*, *B. nigra* or *B. pendula*. A specific birch for use in the processes of the present invention is *B. papyrifera*.

As used herein, " Taxus" or "yew" refers to plants belonging to Taxaceae Gymnopenmae. There are 11 species and five sub-species of Taxus in the world, mainly found in East Asia, North America, and Europe;

"*Echinacea spp.*" Refers to a perennial native to North American which resembles a black-eyed Susan and is called □chinacea, purple coneflower or snake root;

"*Valeriana officinalis*" or "valerian" refers to the plant *Valeriana officinalis* of the valerianaceae family, which is also known as valerian, phu, all-heal, great wild valerian, amantilla, setwall, setewale, capon's tail;

"*Allium sativum*" refers to garlic;

"*Panax ginseng*" refers to ginseng, commonly called Korean ginseng, Chinese ginseng or American ginseng. Asian ginseng is a member of the Araliaceae family, which also includes the closely related American ginseng, Panax quinquefolius, and less similar Siberian ginseng;

"*Eleutherococcus senticosus*," refers to "eleuthero" (which contains eleutheroside A, eleutheroside B (syringin), eleutheroside C, eleutheroside D, eleutheroside E (syringaresinol di-O-β-D-glucoside, liriodendrin), and eleutheroside G, among other constituents);

"Aloe" refers to any of the over 500 different species of Aloe. Aloe Vera is a member of the Lily family and is very-cactus like in its characteristics. This unique plant also belongs to a larger plant family called "Xeroids". Of the 500+ species of Aloe, *Aloe barbadensis miller* (Aloe Vera species) is preferred;

"Vaccinium macrocarpon" refers to cranberry;

"Piper methysticum," a member of the pepper family, refers to a plant native to the South Sea Islands of Micronesia, Melanesia and Polynesia;

"Kola vera," of the family N.O. Sterculiaceae, also known as "Kola nut" refers to the tree that grows about 40 feet high and has yellow flowers spotted with purple; and

"Cinchona," belongs to the family N.O. Rubiaceae and refers to Peruvian bark (Cinchona succirubra) which is an evergreen tree that grows 15 to 45 feet in height.

Plant Components (non-acidic compounds and acidic compounds)

The specific non-acidic compounds and acidic compounds that can be isolated from the plant tissue will depend, in part, upon the specific plant tissue that is being extracted. For example, the bark of Taxus yunnanesis can be extracted employing the methods described herein to provide taxol (paclitaxel) as the non-acidic compound and tannin, fatty acids, and phenols as the acidic compounds;

the needles of the Yew tree can be extracted employing the methods described herein to provide taxol (paclitaxel) as the non-acidic compound, and tannin, fatty acids, and phenols as the acidic compounds;

the root of the Echinacea spp. Can be extracted employing the methods described herein to provide Echinacea extract as the non-acidic compound and tannin, caffeic acid, and cichoric acid as the acidic compounds;

the root of the Valeriana officinalis can be extracted employing the methods described herein to provide Valerian root extract as the non-acidic compound and valerenic acid, isovaleric acid, and tannins as the acidic compounds;

the roots, bark, leaves, or any combination thereof of the Ginkgo biloba can be extracted employing the methods described herein to provide Ginkgolide A, Ginkgolide B, Ginkgolide C, and bilobalide as the non-acidic compounds and tannins, flavonoids (e.g., quercetin, kaempferol, catechin), lignins, shikimic, and succinic acids as the acidic compounds;

the bulb of the Allium sativum can be extracted employing the methods described herein to provide garlic extract as the non-acidic compound and fatty acids and amino acids as the acidic compounds;

the root of the Panax ginseng can be extracted employing the methods described herein to provide Ginseng extract as the acidic compound and tannin, fatty acids, nicotinic acid and pantothenic acid as the acidic compound;

the leaves of the Aloe Vera can be extracted employing the methods described herein to provide aloe gel and barbaloin as the non-acidic compounds and fatty acids, anthraquinones, acidic gelactan, and amino acids as the acidic compounds;

the berries of the Vaccinium macrocarpon can be extracted employing the methods described herein to provide cranberry extract as the non-acidic compounds and benzoic acid, quinic acid, malic acid, citric acid, and hippuric acid as the acidic compounds;

the roots, rhizomes, stems, leaves, or combination thereof of the Eleutherococcus senticosus can be extracted employing the methods described herein to provide Eleutherosides A-G as the non acidic compounds and tannin, fatty acids, and caffeic acid as the acidic compounds;

the rootstock of the Piper methysticum can be extracted employing the methods described herein to provide Kava extract as the non-acidic compounds and tannin, fatty acids, and amino acids as the acidic compounds;

the seeds of the Dill can be extracted employing the methods described herein to provide seed oil as the non-acidic compound and phenolic acids (caffeic acid, ferulic acid, and chlorogenic acid) as the acidic compounds;

the seeds of the Kola nut can be extracted employing the methods described herein to provide kola extract as the non-acidic compounds and tannin and catechins as the acidic compounds; and

the bark of the cinchona (yellow or red) can be extracted employing the methods described herein to provide quinolone alkaloids as the non-acidic compounds and norsoloric acid, tannins, and quinic acid as the acidic compounds.

Table 1 Non-acidic compounds and acidic compounds that can be isolated from specific plant tissue.

Plant	Tissue	Components of Interest (non-acidic)	Acidic Components
Pine Tree	Bark, Needles and Wood	Monoterpenes (careen, pinene	

		and camhora)	
Willow Tree	Bark and Wood		Salicyclic acid acetate (natural aspirin)
Taxus yunnanesis	Bark	Taxol (paclitaxel)	Tannin, fatty acids, phenols
Yew tree	Needles	Taxol (paclitaxel)	Tannin, fatty acids, phenols
Echinacea spp.	Root	Echinacea extract	Tannin, caffeic, cichoric acid, tartaric acid & dicaffeate
Valeriana officinalis	Root	Valerian Roots extract	Valerenic acid, Isovaleric acid, tannins
Ginkgo biloba	Root bark and leaves	Ginkgolide A, B and C, bilobalide	Tannins, flavonoids (quercetin, kaempferol, catechin), lignins, shikimic and succinic acids
Allium sativum (garlic)	Bulb	Garlic extract	Fatty acids, amino acids
Panax ginseng	Root	Ginseng extract	Tannin, fatty acids, nicotinic acid, pantothenic acid
Aloe Vera	Leaves	Aloe gel, barbaloin	Fatty acids, anthraquinones, acidic galactan, amino acids
Vaccinium macrocarpon	Berry	Cranberry extract	benzoic, quinic, malic, citric and hippuric acid
Eleutherococcu	Root, rhizome,	Eleutherosides A-	Tannin, fatty

s senticosus	stems, leaves	G	acids, caffeic acid
Piper methysticum	Rootstock	Kava extract	Tannin, fatty acids, amino acids
Dill	Seeds	Seed oil	Phenolic acids (caffeic, ferulic, chlorogenic)
Kola nut	Seeds	Kola extract	Tannin, catechins
Cinchona (red and yellow)	Bark	Quinoline alkaloids	Cinnamic acid, tannins, quinic acid
Ziziphus (ber, jujube)	Bark		Betulinic acid
Birch (*Betula*)	Bark	Betulin and Lupeol	Betulinic Acid

"Paclitaxel" refers to [2aR-[2aα,4β,4aβ,6β,9α(αR*,βS*),-11,α12α,12aα,12bα]]-β-(Benzoylamino)-α-hydroxybenzenepropanoic acid 6,12b-bis(acetyloxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-4,11,-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1H-cyclodeca[3,4]benz[1,2-b]oxet-9-yl ester.

"Echinacea extract" is believed to include essential oil, polysaccharides, such as inulin, polyacetylenes, betain, glycoside, sesquiterpenes and caryophylene. Echinacea extract is also believed to contain copper, iron, tannins, protein, fatty acids, fat-soluble alkylamides, caffeic acid glycoside (echinacoside), and vitamins A, C, and E.

"*Valeriana officinalis* extract" is a very effective sedative and is used most often to help insomnia, especially due to stress. It has an advantage over prescription sedatives in that it is not habit forming. Valerian has many actions besides its well-known sedative effects. It strengthens the heart and in some cases lower blood pressure. It promotes wound healing and has some antibiotic activity and may be used externally to relieve muscle cramps. It has some expectorant activity and may help a tickly cough. It may actually balance the nervous system helping to calm agitated states and stimulate cases of extreme

fatigue. There are several species of valerian, which vary in potency and can be used similarly, although *V. officinalis* is the preferred plant. Other constituents are a volatile oil, which includes isovalerianic acid and borneol; choline; flavonoids; sterols and several alkaloids, including actinidine, valerianine, valerine, and chatinine. Valepotriates are not water-soluble, but valeric acid is and may be the constituent most likely to produce valerian's sedative effect, especially when used as it was traditionally in water extracts (teas) or water/alcohol extracts (tinctures).

The genus Ziziphus (ber, jujube) belongs to the buckthorn familiy (Rhamnaceae). It is a genus of about 100 species of deciduous or evergreen trees and shrubs distributed in the tropical and subtropical regions of the world. Some species, like Z. mauritiana and Z. jujuba, occur on nearly every continent, whereas other species, like Z. nummularia, Z. spina-christi and Z. mucronata, are restricted in their distribution to distinct areas. Ziziphus species can grow either as trees and shrubs (Z. mauritiana, Z. rotundifolia, Z. jujuba, Z. mucronata) or exclusively as small shrubs or bushes (Z. nummularia, Z. lotus, Z. spina-christi, Z. obtusifolia). This is a tree that may be used for manufacturing betulinic acid.

Many studies have provided clinical evidence that ginkgo prevents many problems throughout the entire body. Ginkgo is gaining recognition as a brain tonic that enhances memory because of its positive effects on the vascular system, especially in the cerebellum. It is also used as a treatment for vertigo, tinnitus (ringing in the ears) and a variety of neurological disorders and circulation problems. Ginkgo may help to counteract the effects of aging, including mental fatigue and lack of energy. Ginkgo has two groups of active substances, flavonoids (a three-ringed molecule with hydroxyl (OH) groups attached) and terpene lactones, including ginkgolides A, B, and C, bilobalide (a sesquiterpene), quercetin (a flavonoid), and kaempferol (a flavonoid). The constituents of gingko include terpenoids (bilobalide), diterpenoids (ginkgolide A, ginkgolide B, ginkgolide C, ginkgolide J, ginkgolide M), flavonoids: flavones (luteolin, tricetin, 2-hydroxyluteolin), biflavones (amentoflavone, ginkgetin, isoginkgetin, sciadoptysin, 5-methoxybilobetin, bilobetin), flavonols (caempherol, quercetin, isorhammetin), flavone glycosides, flavone acyl glycosides, catechins, and steroids (sitosterol, sitosterol glucoside). The

ginkgolides have been shown to control allergic inflammation, anaphylactic shock and asthma. Ginkgo extract is generally derived from dried ginkgo leaves, but also may be derived from gingko root or bark.

"Garlic" contains compounds that are antibacterial, antifungal and reduce blood clotting. In order for the active ingredient that gives garlic its characteristic odor and its therapeutic effects to be released, the garlic clove (or bulb) must be cut or crushed. This releases an enzyme that causes the formation of allicin, the component responsible for garlic's odor and medicinal activity. Active constituents present in garlic include the sulphur compound allicin, produced by crushing or chewing fresh garlic, which in turn produces other sulphur compounds: ajoene, allyl sulfides, and vinyldithiins.

"Ginseng" is believed to increase energy, counter the effects of stress, and enhance intellectual and physical performance. Thirteen ginsenosides have been identified in ginseng, including ginsenosides Rg1 and Rb1. Other constituents include the panaxans, which are believed to help lower blood sugar, and the polysaccharides (complex sugar molecules), which are believed to support immune function. Also, long-term intake may be linked to a reduced risk of cancer.

Applied to wounds, "aloe" is a mild anesthetic, relieving itching, swelling, and pain: it also is antibacterial and antifungal, increases blood flow to wounded areas, and stimulates fibroblasts, the skin cells responsible for wound healing.

"Cranberry" has astringent applications for the urinary tract and is a traditional remedy for bladder infections and kidney-related disorders. Two components of cranberry juice have been shown to inhibit the adherence of *E. coli* to uroepithelial cells. The first is fructose. The second is proanthocyanidin, the chemical structure of which has been elucidated. Fructose inhibits the adherence of type-1 fimbriated *E. coli* and proanthocyanidin inhibits the adherence of P-fimbriated *E. coli* to uroepithelial cells. Cranberry is also a natural diuretic and urinary antiseptic agent.

Although "kava" has undergone much research as to its active ingredients, there is still no definite conclusion. It consists of an oleoresin from which kavalactones originate, starch, sugars, proteins, vitamins B1, B2, B3, B6,

folic acid and E, potassium, manganese, biotin, choline, inositol, fat, glycyrrhizin, lecithin, pantothenic acid, para-aminobenzoic acid, pentacyclic terpenes, phosphorous, and a yellow dye. Kavalactones are considered the most active constituents in the plant. The main use for kava today is in the treatment of anxiety. It is also an excellent muscle relaxant and has diuretic and urinary antiseptic properties, so it may be useful in urinary cystitis and prostatitis. Kava also shows pain-relieving properties.

"Kola vera" or "Cola vera" seeds are said to contain a glucoside, Kolanin (this substance may be a mixture of Kola red and caffeine). The seeds also contain starch, fatty matter, sugar, and a fat decomposing enzyme acting on various oils.

"Dill seed" is an herbal medicine that is used to reduce gas, upset stomach, and colic pains. It is also used to promote the flow of milk in breastfeeding mothers, and to help control bad breath and hiccups. Other names for Dill Seed include: Anethum Graveolens, Dill, and Dillweed.

As used herein, "tannin" refers to tannic acid or gallotannic acid. Tannin varies somewhat in composition, depending on the source, having the approximate empirical formula $C_{76}H_{52}O_{46}$. Tannic acid is a colorless to pale yellow solid; it is believed to be a glucoside in which each of the five hydroxyl groups of the glucose molecule is esterified with a molecule of digallic acid. Tannin is used in tanning animal skins to make leather; it transforms certain proteins of animal tissue into compounds that resist decomposition. It is also used in manufacturing inks, as a mordant in dyeing, and in medicine as an astringent and for treatment of burns.

As used herein, "fatty acids" refers to a long-chain of carboxylic acids that may either be saturated (without double bond) or non-saturated (with double bond). It refers to any acid derived from fats by hydrolysis (e.g., oleic acid, palmitic acid, or stearic acid); any long–chain monobasic organic acid.

As used herein, "phenols" refers to compounds that include a C_6H_5OH backbone. They are aromatic alcohols that are optionally substituted by one or more substituents. Phenols exhibits weak acidic properties and are sometimes called carbolic acids, especially when in water solution.

As used herein, "caffeic acid" refers to 3-(3,4-Dihydroxyphenyl)-2-propenoic acid.

As used herein, "valeric acid" refers to pentanoic acid; valerianic acid; and propylacetic acid.

As used herein, "isovaleric acid" refers to 3-Methylbutanedioic acid and isovalerianic.

As used herein, "flavonoid" refers to polyphenols that have a carbon skeleton. They have an acidic nature due to the phenol groups.

As used herein, quercetin refers to 2-(3,4-Dihydroxyphenol)-3,5,7-trihydroxy-4H-1-benzopyran-4-one.

As used herein, "kaempferol" refers to 3,5,7-Trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one.

As used herein, "catechin" refers to (2R-trans)-2-(3,4-dihydroxyphenyl)-3,-4-dihydro-2H-1-benzopyran-3,5,7-triol.

As used herein, "lignin" refers to a highly polymerized and complex chemical compound especially common in woody plants. The cellulose walls of the wood become impregnated with lignin, a process called lignification, which greatly increases the strength and hardness of the cell and gives the necessary rigidity to the tree. It is believed to be essential to woody plants for them to stand erect.

As used herein, "amino acids" refers to any one of a class of simple organic compounds containing carbon, hydrogen, oxygen, nitrogen, and in certain cases sulfur. These compounds are the building blocks of proteins. They are characterized by the presence of a carboxyl group (COOH) and an amino group (NH_2). The 20 amino acids commonly found in animals are alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. More than 100 less common amino acids also occur in biological systems, particularly in plants. Every amino acid except glycine can occur as either of two optically active stereoisomers, D or L; the more common isomer in nature is the L-form. When the carboxyl carbon atom of one amino acid covalently binds to the amino nitrogen atom of another amino acid with the release of a water molecule, a peptide bond is formed.

As used herein, "shikimic acid" refers to [3R-(3α,4α,5β]-3,4,5-Trihydrooxy-1-cyclohexene-1-carboxylic acid.

As used herein, "succinic acid" refers to butanedioic acid ($HOOCCH_2CH_2COOH$).

As used herein, "nicotinic acid" refers to 3-Pyridinecarboxylic acid.

As used herein, "pantothenic acid" refers to "(R)-N-(2,4-Dihydroxy-3,3-dimethyl-1-oxobutyl)-β-alanine.

As used herein, "anthraquinone" refers to 9,10-anthracenedione.

As used herein, "acidic galactan" refers to a poly sugar with attached carboxylic groups.

As used herein, "benzoic acid" refers to benzoic acid, $C_6H_5CO_2H$. It is the simplest aromatic carboxylic acid. In addition to being synthesized from a variety of organic compounds (e.g., benzyl alcohol, benzaldehyde, toluene, and phthalic acid), it may be obtained from resins, notably gum benzoin. It is used largely for making its salts and esters, most notably sodium benzoate, which is widely used as a preservative in foods and beverages and as a mild antiseptic in mouthwashes and toothpastes.

As used herein, "quinic acid" refers to [1R-(1α,3α,4α,5β]-1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid.

As used herein, "malic acid" refers to hydroxybutanedioic acid.

As used herein, "citric acid" refers to citric acid or 2-hydroxy-1,2,3-propanetricarboxylic acid, $HO_2CCH_2C(OH)(CO_2H)CH_2CO_2H$, an organic carboxylic acid containing three carboxyl groups. It is responsible for the tart taste of various fruits in which it occurs, e.g., lemons, limes, oranges, pineapples, and gooseberries.

As used herein, "hippuric acid" refers to N-Benzoylglycine.

As used herein, "ferulic acid" refers to 3-(4-Hydroxy-3-methoxyphenyl)-2-propenoic acid.

As used herein, "chlorogenic acid" refers to [1S-(1α,3β,4α,5α]-3-[[3-(3,4-Dihydroxyphenyl)-1-oxo-2-propenyl]oxy]-1,4,5,trihydroxycyclohexanecarboxylic acid.

As used herein, "cinnamic acid" refers to 3-phenyl-2-propenoic acid.

Betulinic acid can also be obtained from Tanzanian Tree Uapaca nitida Mull-Arg (*Euphorbiacea)*, leaves and bark of *Bacopa monniera*(West Bengal), all species of *Dilleniacea (Acrotrema arnothianum* Wight, *Dillenia andamanica* Parkinson, *D. aurea* Smith, *D. bracteata* Wight, *D. indica* Linn, D. pentagina Roxb, *D. retusa* Thunb, *D. scabtalla* (D. Don) Roxb, exWall, *Tetracera* (Houtt. exChrism.& Panz., Merr.), *Tetracera akara* (Burm.f.) Merr., *T. indica* (Houtt. exChrism.& Panz., Merr.), *T. sarmentosa*(L.) Vahl. Subsp. Andamanica (Hoogl.) Hoogl., and *T. scandens* (L.) Merr.

As used herein, "separating" refers to the process of removing solids from a mixture. The process can employ any technique known to those of skill in the art, e.g., decanting the mixture, filtering the solids from the mixture, or a combination thereof.

As used herein, "filtering" refers to the process of removing solids from a mixture by passing the liquid through a filter, thereby suspending the solids on the filter.

As used herein, "decanting" refers to the process of pouring off a liquid without disturbing the sediment, or the process of pouring off a liquid with a minimal disturbance of the sediment.

As used herein, "aqueous base" refers to a solution of water, and a substance that produces OH^- ions in the aqueous solution. Specifically, the aqueous base can include water and at least one of a lithium ion (Li^+), a sodium ion (Na^+), a potassium ion (K^+), a calcium ion (Ca^{2+}), and a barium ion (Ba^+). More specifically, the aqueous base can include water and at least one of sodium hydroxide (NaOH) and potassium hydroxide (KOH).

As used herein, "alkaline metal" refers to metals of Group IA of the Periodic Table of Elements, e.g., sodium (Na) and potassium (K).

As used herein, "alkaline earth metal" refers to metals of Group IIA of the Periodic Table of Elements, e.g., magnesium (Mg) and calcium (Ca).

The present invention employs a solvent that: (i) is water-immiscible, (ii) is capable of forming an azeotropic mixture with water, or (iii) has a boiling point of at least 100°C. In one embodiment, the solvent is water-immiscible. In another embodiment, the solvent is capable of forming an azeotropic mixture with water. In another embodiment, the solvent has a boiling point of at least

100°C. In another embodiment, the solvent is water-immiscible and is capable of forming an azeotropic mixture with water. In another embodiment, the solvent is water-immiscible and has a boiling point of at least 100°C. In another embodiment, the solvent is capable of forming an azeotropic mixture with water and has a boiling point of at least 100°C.

In one embodiment, the solvent having a boiling point of at least 100°C can have a boiling point of at least about 120°C, at least about 140°C, or at least about 160°C.

It is appreciated that those of skill in the art understand that the solvent should not chemically react with any of the starting materials or reagents present in the reaction mixture, to any significant degree, under the reaction conditions employed. For example, the solvent should not react with the alkaline present at the elevated temperatures typically employed during the heating step.

As used herein, "water-immiscible solvent" refers to a solvent that is not miscible (i.e., not capable of mixing in all proportions) with water. Suitable specific water-immiscible solvents include, e.g., aromatic hydrocarbons such as xylenes, *o*-xylene, *m*-xylene, *p*-xylene, toluene, benzene, and combinations thereof; chlorinated solvents such as chloroform and methylene chloride; as well as other organic solvents such as ethyl-*tert*-butyl ether and ethyl acetate.

As used herein, "water-miscible solvent" refers to a solvent that is miscible (i.e., is capable of mixing in all proportions) with water. Suitable specific water-miscible solvents include, e.g., methanol, ethanol, *iso*-propanol, *tert*-butanol, ethylene glycol, acetone, 1-propanol and propylene glycol.

As used herein, "distill" or "distillation" refers to the process of extracting the volatile components of a mixture by the condensation and collection of the vapors that are produced as the mixture is heated. The process includes the evaporation and subsequent collection of a liquid by condensation.

As used herein, "concentrating" or "condensing" refers to the process whereby the volume is reduced, by the removal of liquid.

As used herein, "mother liquor" refers to the liquid obtained after solids are removed from a mixture or a solution of solids in a liquid. As such, the mother liquor will not include an appreciable amount of these solids.

As used herein, "birch" refers to any of the several deciduous trees of the genus *Betula*. The birches comprise the family *Betulaceae* in the order *Fagales*. Birch trees include, for example, white birch, *B. alba*; sweet, black or cherry birch, *B. lenta*; monarch birch, *B. Maximowicziana*; dwarf or arctic birch, *B. Nana*; Japanese white birch, *B. Platyphyla Japonica*; smooth-bark birch, *B. Pubescens*; yellow birch, *B. alleghaniensis*; paper, white or canoe birch, *B. papyrifera*; grey birch, *B. populifolia*; river birch, *B. nigra*; and the European birches, *B. pubescens*; *B. Alba* and *B. pendula*. Specifically, the birch can be *B. alba*, *B. lenta*, *B. Maximowicziana*, *B. Nana*, *B. Platyphyla Japonica*, *B. Pubescens*, *B. alleghaniensis*, *B. papyrifera*, *B. populifolia*, *B. nigra*, *B. pubescens*, *B. Alba* or *B. pendula*. A specific birch for use in the processes of the present invention is *B. papyrifera*.

As used herein, "birch bark" refers to inner birch bark and outer birch bark. Inner birch bark is more dense and granular than outer birch bark, while outer birch bark is more flexible and fibrous than inner birch bark. Outer birch bark is light in color, thin (1-5 mm), tough, and of low water-content relative to inner birch bark. The inner bark is darker in color, thicker (3-10 mm) and non-fibrous relative to the outer bark. The inner bark is the portion of the tree wherein significant water transport occurs (i.e., an area of high water content). Due to the differences in the physical properties of inner birch bark and outer birch bark, fragmentation produces outer birch bark shreds and inner birch bark chunks.

Outer birch bark shreds can be separated from the inner birch bark chunks using any suitable means. The separation can conveniently be accomplished by screening the mixture through a mesh having openings intermediate in size between the smaller inner bark chunks and the larger outer bark shreds. The smaller inner bark chunks fall through the screen and are separated from the outer bark.

The "mesh" can be a unit comprising one or more open spaces in a cord, thread, or wire network in which the cords, threads or wires surround the spaces. Any mesh suitable to separate inner birch bark from outer birch bark can be employed. Typically, the mesh is a wire mesh containing openings of about ½ of an inch by ½ of an inch, or smaller. For example, mesh can conveniently

contain openings of about 1/4 of an inch by about 1/4 of an inch. Specifically, the size of the mesh can be about 20 mm by about 20 mm, or about 10 mm by about 10 mm, or about 6 mm by about 6 mm. More specifically, the size of the mesh can be about 3 mm by about 3 mm.

Alternatively, the inner birch bark chunks and outer birch bark shreds may be separated with the use of an air classifier. As used herein, an "air classifier" is a device which operates on the principle of the differing properties of the two components (e.g., inner and outer birch bark) in an air stream to effect a physical separation. Typically, the less dense outer bark travels a greater distance in the air stream than the more dense inner bark. The inner bark, along with other materials, falls rapidly from the stream of air. As a result, the inner birch bark and the outer birch bark can be separated.

After separating outer birch bark from inner birch bark, outer birch bark of about 10 wt.% to about 45 wt.% based on initial birch bark content is typically obtained and inner birch bark of about 55 wt.% to about 85 wt.% is typically obtained.

For use in the processes of the present invention, birch bark shreds less than about 10 mm in diameter can conveniently be used. More specifically, outer birch bark shreds less than about 6 mm in diameter, less than about 4 mm in diameter, or less than about 2 mm in diameter, can be used. Alternatively, birch bark pellets of about 5.0 mm in length by about 4 mm in diameter, or about 2.5 mm in length by about 2 mm in diameter can conveniently be used. In addition, bark pellets of about 0.25 kg/liter to about 1.0 kg/liter, or about 0.5 kg/liter to about 0.7 kg/liter can conveniently be used.

As used herein, "aromatic hydrocarbon" refers to a compound having at least one phenyl or naphthyl ring, wherein the compound contains carbon and hydrogen atoms. The aromatic hydrocarbon can optionally be substituted, e.g., with one or more groups selected from the group of alkyl (e.g., methyl), hydroxyl, halo, alkoxy, cyano, carboxyl, sulfonyl, and amino. Suitable specific aromatic hydrocarbons include, e.g., xylenes, *o*-xylene, *m*-xylene, *p*-xylene, toluene, benzene, and combinations thereof.

As used herein, "reflux" refers to the process of boiling a liquid in a vessel attached to a condenser so that the vapors continuously condense for reboiling.

As used herein, "washing" refers to the process of purifying a solid mass (e.g., crystals) by passing a liquid over and/or through the solid mass, as to remove soluble matter. The process includes passing a solvent, such as distilled water, over and/or through a precipitate obtained from filtering, decanting, or a combination thereof. For example, in one embodiment of the invention, washing includes contacting solids with water, vigorously stirring (e.g., for two hours), and filtering. The solvent can be water, can be an aqueous solvent system, or can be an organic solvent system. Additionally, the washing can be carried out with the solvent having any suitable temperature. For example, the washing can be carried out with the solvent having a temperature between about 0°C and about 100°C.

As used herein, a "binder" refers to a substance that can effectively bind (i.e., chemically attach itself, physically attach itself and/or chemically react) with selective compound(s) present in a mixture. It is believed that substances such as aluminum alcoholates bind strongly and irreversibly to triterpenoid or triterpene acids and tannins, therefore providing complete discoloration of crude birch bark extract. Suitable binders useful in the present invention include, e.g., metal hydrides (e.g., lithium hydride (LiH), sodium hydride (NaH), potassium hydride (KH), calcium hydride (CaH_2), and lithium aluminum hydride ($LiAlH_4$); metal alcoholates (e.g., sodium methoxide (NaOMe), sodium ethoxide (NaOEt), potassium methoxide (KOMe), potassium ethoxide (KOEt), aluminum *iso*-propoxide [$Al(i\text{-}OPr)_3$], aluminum *tert*-butoxide [$Al(t\text{-}OBu)_3$], and aluminum methoxide [$Al(OMe)_3$]), ortho-esters (e.g., ethylorthocarbonate), dialkoxysulfates (e.g., dimethylsulfate and diethylsulfate), alumina and silica.

As used herein, "metal hydride" refers to a binary compound of hydrogen and a metal. Suitable metal hydrides include e.g., lithium hydride (LiH), sodium hydride (NaH), potassium hydride (KH), calcium hydride (CaH_2), and lithium aluminum hydride ($LiAlH_4$).

As used herein, "metal alcoholate" or "alcoholate" refers to an organic alcohol wherein the hydroxy hydrogen has been replaced with a metal, e.g.,

$(CH_3CH_2O)_3Al$. Aluminum alcoholates are suitable reagents for triterpene purification because it is believed that aluminum alcoholates bind strongly and irreversibly to acids and tannins, therefore providing complete discoloration of the total extract. Suitable specific metal alcoholates include, e.g., sodium methoxide (NaOMe), sodium ethoxide (NaOEt), potassium methoxide (KOMe), potassium ethoxide (KOEt), aluminum *iso*-propoxide [Al(i-OPr)$_3$], aluminum *tert*-butoxide [Al(t-OBu)$_3$], and aluminum methoxide [Al(OMe)$_3$].

As used herein, "hydrolyze" or "hydrolysis" refers to the process of converting a carboxylic ester to the corresponding carboxylic acid, with the addition of water. The reaction (i.e., hydrolysis) can conveniently be carried out employing any suitable reagent(s) and reaction condition(s). For example, the reaction can be carried out in a suitable solvent and at a suitable temperature and pressure, under basic conditions, neutral conditions, or acidic conditions. Suitable reagents and reaction conditions are disclosed, e.g., in Advanced Organic Chemistry, Part B: Reactions and Synthesis, Carey and Sundberg, Plenum Press; Comprehensive Organic Transformations, Larock, Wiley & Sons; and Advanced Organic Chemistry, Reactions Mechanisms, and Structure, March, McGraw Hill.

For example, the hydrolysis can be carried out at a pH of greater than about 7.0 (e.g., a pH of about 7-8, 8-9, 9-10, 10-11, or 11-12). When the hydrolysis is carried out at a pH of greater than 7.0, one or more suitable bases will typically be employed. Suitable bases include metal hydroxides and metal alkoxides. Suitable metal hydroxides include sodium hydroxide, potassium hydroxide, lithium hydroxide, calcium hydroxide and barium hydroxide. Suitable metal alkoxides include lithium methoxide, lithium ethoxide, lithium isopropoxide, lithium tert-butoxide, sodium methoxide, sodium ethoxide, sodium isopropoxide, sodium tert-butoxide, potassium methoxide, potassium ethoxide, potassium isopropoxide, potassium tert-butoxide, magnesium methoxide, magnesium ethoxide, barium methoxide, barium ethoxide, calcium methoxide and calcium ethoxide. A specific base suitable for the processes of the present invention (i.e., hydrolysis of birch bark) is sodium hydroxide.

Alternatively, the hydrolysis can be carried out at a pH of less than about 7.0 (e.g., a pH of about 1-2, 2-3, 3-4, 4-5, 5-6, or 6-7). When the hydrolysis is

carried out at a pH of less than about 7.0, one or more suitable acids will typically be employed. Suitable acids include, for example, hydrochloric acid, phosphoric acid, formic acid, hydrobromic acid, sulfuric acid, nitric acid, acetic acid, and combinations thereof. A specific acid suitable for the processes of the present invention (i.e., hydrolysis of birch bark) is sulfuric acid.

Alternatively, the hydrolysis can be carried out at a pH of about 6.5 to about 7.5 (i.e., neutral conditions). When the hydrolysis is carried out at about 6.5 to about 7.5 (i.e., neutral conditions), water at standard pressure or at an elevated pressure can be employed.

As used herein, "natural ester" refers to an organic compound (e.g., triterpenes or triterpenoids) having at least one carboxylic ester group.

As used herein, "azeotropic distillation" refers to the process of boiling off or distilling any liquid mixture having constant minimum and maximum boiling points and distilling off without decomposition and in a fixed ratio, as with benzene and water, or as with toluene and water. In a specific embodiment, the azeotropic distillation can include the co-distillation of water and at least one of xylenes, *o*-xylene, *m*-xylene, *p*-xylene, toluene, and benzene. Such a distillation can be carried out at a temperature and a period of time, effective to distill or boil more than about 90% of the water, more than about 95% of the water, more than about 98% of the water, or up to about 100 wt.% of the water. With the distillation or boiling off of the water, it is appreciated that those of skill in the at recognize that a discrete amount of the organic solvent (e.g., xylenes, *o*-xylene, *m*-xylene, *p*-xylene, toluene, and/or benzene) will also distill or boil off with the water.

As used herein, "azeotropic mixture" refers to a mixture which would undergo azeotropic distillation.

As used herein, "agitating" refers to the process of putting a mixture into motion with a turbulent force. Suitable methods of agitating include, e.g., stirring, mixing, and shaking.

As used herein, "precipitating" refers to the process of causing a solid substance (e.g., crystals) to be separated from a solution. The precipitating can include, e.g., crystallizing.

As used herein, "polar organic solvent" refers to an organic solvent having a measurable dipole. Specifically, it refers to an organic solvent having a dielectric constant of at least about 15, at least about 20, or between about 20 and about 30.

As used herein, "non-polar organic solvent" refers to an organic solvent having no measurable dipole. Specifically, it refers to an organic solvent having a dielectric constant of less than about 15, less than about 10, or between about 6 and about 10.

As used herein, "drying" refers to the removal of water and/or solvent, such that the water and/or solvent content is below about 5 wt.%, below about 2 wt.% or below about 1 wt.%.

As used herein, "neutralizing" refers to the process of changing or bringing the pH to about 7 ± 1. As such, the neutralizing can include bringing the pH to about 6 to about 8. In a specific embodiment, the neutralizing can include bringing the pH to about 6.5 to about 7.5 (i.e., to a pH of about 7 ± 0.5). In yet another specific embodiment, the neutralizing can include bringing the pH to about 6.75 to about 7.25 (i.e., to a pH of about 7 ± 0.25).

As used herein, "acidifying" refers to the process of lowering the pH to below 7.0. For example, in one specific embodiment, the acidifying includes lowering the pH to below about 6.0. In another specific embodiment, the acidifying includes lowering the pH to below about 5.0. In yet another specific embodiment, the acidifying includes lowering the pH to below about 4.0.

As used herein, "purifying" refers to the process of ridding a solid substrate (e.g., crystals) of impurities. Suitable methods of purifying include, e.g., washing, recrystallizing and drying.

In cases where compounds are sufficiently basic or acidic to form stable nontoxic acid or base salts, administration of the compounds as salts may be appropriate. Examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids, which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, α-ketoglutarate, and α-glycerophosphate. Suitable inorganic salts may also be formed, including hydrochloride, sulfate, nitrate, bicarbonate, and carbonate salts.

Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids can also be made.

The compositions that include a compound obtained by the processes described herein, can be formulated as pharmaceutical compositions and/or cosmetic compositions, and administered to a mammalian host, such as a human patient in a variety of forms adapted to the chosen route of administration, i.e., orally or parenterally, by intravenous, intramuscular, topical or subcutaneous routes.

Thus, the present compositions can be systemically administered; e.g., orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the compositions may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such preparations should contain at least 0.1% of the triterpene compound. The percentage of the compositions can, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of active compound (i.e., triterpene compound) in such therapeutically useful compositions is such that an effective dosage level will be obtained.

The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or

a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the active compound (i.e., triterpene), sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the active compound (i.e., triterpene) may be incorporated into sustained-release preparations and devices.

The composition may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the triterpene can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient, which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of

the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the triterpene in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying techniques, which yield a powder of the triterpene, plus any additional desired ingredient present in the previously sterile-filtered solutions.

For topical administration, the present compositions may be applied in pure form, i.e., when they are liquids. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid.

Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Useful liquid carriers include water, alcohols or glycols or water-alcohol/glycol blends, in which the triterpene can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers.

Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

Examples of useful dermatological compositions which can be used to deliver the compositions of the triterpene, to the skin, are known to the art; for example, see Jacquet et al. (U.S. Pat. No. 4,608,392), Geria (U.S. Pat. No. 4,992,478), Smith et al. (U.S. Pat. No. 4,559,157) and Wortzman (U.S. Pat. No. 4,820,508).

Useful dosages of the compositions of the triterpene can be determined by comparing their in vitro activity, and in vivo activity in animal models.

Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949.

Generally, the concentration of the compositions of the triterpene in a liquid composition, such as a lotion, will be from about 0.1-25 wt-%, preferably from about 0.5-10 wt-%. The concentration in a semi-solid or solid composition such as a gel or a powder will be about 0.1-5 wt-%, preferably about 0.5-2.5 wt-%.

The amount of the triterpene, required for use in treatment will vary not only with the particular salt selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician.

In general, however, a suitable dose will be in the range of from about 0.5 to about 100 mg/kg, e.g., from about 10 to about 75 mg/kg of body weight per day, such as 3 to about 50 mg per kilogram body weight of the recipient per day, preferably in the range of 6 to 90 mg/kg/day, most preferably in the range of 15 to 60 mg/kg/day.

The composition is conveniently administered in unit dosage form; for example, containing 5 to 1000 mg, conveniently 10 to 750 mg, most conveniently, 50 to 500 mg of triterpene per unit dosage form.

Ideally, the composition should be administered to achieve peak plasma concentrations of the triterpene of from about 0.5 to about 75 μM, preferably, about 1 to 50 μM, most preferably, about 2 to about 30 μM. This may be achieved, for example, by the intravenous injection of a 0.05 to 5% solution of the triterpene, optionally in saline, or orally administered as a bolus containing about 1-100 mg of the triterpene. Desirable blood levels may be maintained by continuous infusion to provide about 0.01-5.0 mg/kg/hr or by intermittent infusions containing about 0.4-15 mg/kg of the triterpene(s).

The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, e.g., into a number of discrete loosely spaced administrations; such as multiple inhalations from an insufflator or by application of a plurality of drops into the eye.

The ability of a composition of the invention to act, e.g., as an anti-fungal agent, anti-bacterial agent, and/or anti-viral agent may be determined using pharmacological models which are well known to the art.

The compositions of the invention may be also be useful as pharmacological tools for the further investigation of the mechanism of their anti-fungal, anti-bacterial, and/or anti-viral action.

The compositions of the invention can also be administered in combination with other therapeutic agents that are effective to treat, e.g., fungal infections, bacterial infections, and/or viral infections; or to inhibit or kill a fungus, bacterium, and/or virus.

References in the specification to "one embodiment", "an embodiment", "an example embodiment", etc., indicate that the embodiment described may include a particular feature, structure, or characteristic, but every embodiment may not necessarily include the particular feature, structure, or characteristic. Moreover, such phrases are not necessarily referring to the same embodiment. Further, when a particular feature, structure, or characteristic is described in connection with an embodiment, it is submitted that it is within the knowledge of one skilled in the art to affect such feature, structure, or characteristic in connection with other embodiments whether or not explicitly described.

Referring to **Figure 1**, birch bark processing (**1**) utilizing distillation (**6**) is illustrated. The processing (1) provides for the isolation of betulinic acid (**15**), betulin (**17**), and/or lupeol (**20**). The processing includes extracting (**3**) outer birch bark (**2**) with an organic solvent to provide a birch bark extract (**4**). Suitable solvents, techniques and conditions for the extracting (**3**) are known to those of skill in the art. The extracting (**3**) will preferably employ suitable solvent(s) in which the desired compound(s) are relatively soluble. The extracting (**3**) can be carried out under conditions (e.g., temperature, pressure and period of time), effective to extract the desired compound(s) in a relatively high yield, while minimizing any decomposition of the desired compound(s).

The extracting (**3**) effectively provides birch bark extract (**4**), which can subsequently be refluxed (**21**) with a metal hydroxide (**5**), distilled (**6**), and filtered/decanted (**7**), to provide the solids (**8**) and filtrate (**9**). The refluxing (**21**) of the birch bark extract (**4**) with the metal hydroxide (**5**) can be carried out

under conditions (e.g., temperature and period of time) and with reagents and solvents, effective to reflux (21) the reaction mixture, while minimizing any decomposition of the desired compound(s). Suitable metal hydroxides (5) are known to those of skill in the art.

The reaction mixture can then be distilled (6), to effectively remove water present in the reaction mixture. This distillation (6) will remove not only water, but also a discrete amount or organic solvent (e.g., xylenes, toluene and/or benzene) present in the reaction mixture. The distillation (6) will also not remove any appreciable amounts of metal hydroxides (5) along with the water. Additionally, no appreciable amounts of triterpenes or triterpenoids are removed with the water during the distillation (6). It is believed that the metal hydroxide reacts with acids (e.g., triterpenoid acids) while in solution, to provide the corresponding salts. These salts are relatively insoluble in the organic solvent employed. As such, the salts will precipitate from the solution after removal of the water during the distillation (6). The solids (8), which can be visible as a black tar, precipitates from solution, and can subsequently be separated (e.g., filtered or decanted (7)) from solution to provide the solid (8) and filtrate (9). As discussed below, the solid (8) contains betulinic acid (15), and can be purified accordingly.

The filtrate (9) can be treated with binder (25) to provide a mixture. The mixture can be filtered (27) to provide a second filtrate. The second filtrate can be concentrated (29), and crystallized (30), to provide betulin (17) and a filtrate (16). This filtrate (16) can be concentrated (31), washed (33), crystallized (35), concentrated (36) and crystallized (37) to provide lupeol (20).

The solid (8) described above can be washed with water (38), neutralized/acidified (10), filtered/decanted (11), and subsequently crystallized (12) to provide betulinic acid (15).

Referring to **Figure 2**, plant matter processing (100) utilizing distillation (106) is illustrated. Plant matter (101) can be purified, to provide acidic components of plant mater extract (117) and non-acidic components of plant matter extract (120). Specifically, the plat matter (101) is extracted (102) to provide plant matter extract (103). The plant matter extract (103) can be refluxed with a metal hydroxide (105), distilled (106), and filtered/decanted

(107), to provide solids (108) and filtrate (109). The solids (108) can be washed (130), neutralized/acidified (110), filtered/decanted (111), and crystallized (112), to provide acidic components of plant matter extract (117). The filtrate (109) can be contacted with binder (131), filtered (132), concentrated (113), and crystallized (114), to provide filtrate (115) and non-acidic component of plant matter extract (116). The filtrate (115) can be concentrated (140), washed (141), and crystallized (142) to provide non-acidic component of plant matter extract (120).

The present invention will be described by the following examples. The examples are for illustration purposes and do not otherwise limit the invention.

Examples

Example 1: Extraction of outer birch bark with xylenes

Air-dry pellets of outer birch bark (200 g) were extracted with p-xylene (1 x 1 L) and then with p-xylene (3 x 300ml). Each time the extraction mixture was refluxed for 20 min and then filtered at 90°C. The solvent of the combined organic extracts was evaporated and the residue was dried at 80°C, *in vacuum*, to give 44 g (yield is 22 wt.%) of dry birch bark extract. The p-xylene extract was used without further purification.

Example 2: Isolation of betulin

The birch bark extract (44 g) obtained from the procedure in Example 1 was dissolved in xylenes (1.1 L) at 80°C. Sodium hydroxide (NaOH) (8.8g) was dissolved in 80°C water (17.4 ml). The two mixtures were combined, vigorously stirred and refluxed for 1 hr. Water (17.4 ml) was removed from the reaction mixture by azeotropic distillation on a Dean-Stark trap, and the reaction mixture was filtered to remove the black tar that precipitated from solution. This black tar includes the crude sodium salts of triterpenoid acids (19.8 g), including betulinic acid, as shown in Example 4 below. To the mother liquor xylene solution obtained after filtration was added aluminum *iso*-propoxide [$Al(Oi\text{-}Pr)_3$] (2.64 g, 12.9 mmol) and stirred for 1hr at 85°C. Alumina oxide (Al_2O_3) (4.4 g, acidic Brockman I) was added and the reaction mixture was stirred for 4 hrs at 85°C, filtered and 800 ml of xylenes was evaporated. The resulting solution was

cooled to room temperature and left undisturbed for 10 hrs. The crystals were filtered, dried and 21 g (47.7 wt.%, based on the birch bark extract) of betulin (98% purity) was obtained. The concentration of betulinic acid in this betulin was less than 0.02 wt.% by GC/MS and HPLC analysis.

Example 3: Isolation of lupeol

The mother liquid from Example 2 was evaporated to afford 11 g (25 %) of crude lupeol / betulin mixture. 20 ml of acetone was added to this mixture, stirred at 60°C for one hour and cooled down 5-10°C for two hours. The yellowish precipitate (4.5 g) was filtered and crystallized from xylene. The mother liquid after xylene filtering was removed and the yellowish solid crystals of lupeol were crystalyzed from cyclohexane. The mother liquid after cycloxehane filtering was removed and the solids were washed with 5 ml of acetone. 1.1g of white lupeol crystals (98%+ purity) were obtained (yield is 2.5 wt.%, based on birch bark extract).

Example 4: Isolation of betulinic acid

19.8 g the sodium salts of triterpenoid acids obtained in Example 2 above was washed with water (4 x 100ml). The solid after filtration was acidified with a 5% solution of HCl in water (to pH=5), filtered and dried to give 2.5 g of crude product. The crude product was then dissolved in 80 ml of *iso*-propanol (i-PrOH), and 65 ml of *iso*-propanol (i-PrOH) was evaporated and filtered at 35°C. The crystals were dried to provide 1.3 g (3 wt.%, based on birch bark extract) of betulinic acid (90 % purity). After a second crystallization from *iso*-propanol (i-PrOH) 0.9 g (2.0 wt.%, based on birch bark extract) of betulinic acid (99% purity) was obtained.

Example 5: Azeotropic distillation sing sodium hydroxide, water and xylenes

A solution of NaOH (90g) in 180 ml of water was added to the vigorously stirred solution of birch bark extract in xylenes* (13.5 L) at 90°C. The stirred solution was kept at 100°C for 1 hour. Azeotropic mixture of water and xylenes (total volume 4.0 L) was distilled (xylene recycling). The xylenes solution (9.5 L) was then filtered at 90-100°C into the heated up to 100°C reactor

and transferred to example 7.

The black solid product was dried at 80°C for 5 hours in vacuum. This product (196g) was transferred to example 9 for betulinic acid separation and purification.

* - The cheapest Xylene isomers mixture can be used for this purpose (Aldrich 24,764-2; 18 L, $93.80).

Example 6: Azeotropic Distillation

A solution of KOH (126g in 180 ml of water) was added to the vigorously stirred solution of birch bark extract (456 g in 13.5 L of xylenes*) at 90°C. The stirred solution was kept at 100°C for 1 hour. Azeotropic mixture of water and xylenes (total volume 4.0 L) was removed (*xylene recycling*). The solution was then filtered at 90-100°C.

The black solid product was transferred from filter and dried at 80°C for 5 hours in vacuum. This product (221g) was transferred to example 9 for betulinic acid separation and purification.

* - The cheapest Xylene isomers mixture may be used for this purpose (Aldrich 24,764-2; 18 L, $93.80)

Example 7: Treatment with Dimethyl Sulfate and crystallization

Recycled betulin (33 g) and 18.0 g of K2CO3 were added to the stirred hot filtrate of treated with NaOH birch bark extract (350 g) in xylenes (9.5 L) (xylenes solution from example 5). This mixture was stirred for 10 minutes at 100°C. Dimethyl sulfate (12 ml) was added and the resulting mixture was stirred for 2 hrs. Then 180 ml of water was added and mixture was stirred at 100°C for 0.5 hour. Xylenes (5.5 L) and water (170 ml) were removed by azeotropic distillation. The remain hot xylenes solution was filtered and left for crystallization at 10°C for 3 hours. The precipitate was filtered, washed with cold xylenes (2 times x 375 ml) and dried. The yield of 96%+ purity betulin was 261.4g (13%). The filtrate solution was used in Example 8 for Lupeol separation.

Example 8: Lupeol separation and betulin recycling

Xylenes were evaporated from and the filtrate solution (the filtrate solution from example 7). Acetone (0.5 L) was then added and the mixture was vigorously stirred and refluxed for 1 hr. Then acetone solution was cooled down to room temperature and filtered. Operation with acetone was repeated three times.

White solid material (83 g) after washing with acetone was dried and crystallized from 1.8 L of xylene. After filtration 37 g of solid white material (75% of betulin and 10% of lupeol, 15% others) was received and used in Example 7 as recycling betulin product. The filtrate after crystallization was evaporated and the residue was crystallized from xylenes (0.8 L).

Xylenes filtrate was evaporated and the yellowish solid material (65% ± 5% purity of lupeol, 37 g) was obtained after drying at 100°C. Lupeol of 65% ± 5% purity was added to acetone (800 ml) and refluxed until all lupeol dissolves. Temperature of the reaction mixture was then decreased to room temperature and the reaction mixture was kept at room temperature until crystallization was completed (about 12 hrs). After filtration the crystals of lupeol were crystallized again using the same procedure from acetone (150 ml) to give 9.4 g of lupeol (85% purity). The yield of lupeol is 0.47% calculated from starting outer birch bark.

Combined acetone mother liqueurs were evaporated. Yellow solid (27.6 g) product (50% of lupeol) was obtained and can be used for lupeol separation in recycling process.

Example 9: Betulinic acid separation and purification

Dry black solid product from step 1 (196 g) was stirred in 3.5 L of water and filtered. This operation was repeated two times using 1 L of water each time. A solution of 2% HCl (0.8 L) was then added to a suspension of solid product in 250 ml of water and stirred for 0.5 hours, filtered. Solid product was then washed with water (2x200 ml) and dried at 40°C for 5 hours in vacuum drier to give 32 g of crude betulinic acid.

Crude betulinic acid (32 g) was dissolved in 0.95 L of refluxed 2-propanol, filtered and evaporated to 300 ml, and left for crystallization at 35°C for 5 hours. Precipitate was filtered at 35°C and dried at 40°C for 5 hours in

vacuum drier to give 17.6 g of crude betulinic acid. Crude betulinic acid (17.6 g) was refluxed with 500 ml of 2-propanol and filtered. 2-Propanol was evaporated to volume of 170 ml. After crystallization at room temperature white crystals were filtered and 15.5 g of betulinic acid (92 %+ purity) was obtained.

Betulinic acid (15.5 g) was dissolved in 500 ml of refluxed xylenes and a solution of NaOH (5 g) in 10 ml of water was added. This solution was refluxed with a good stirring for 1 hour. Xylenes and water (total volume is 90 ml) were removed by azeotropic distillation from reaction mixture. Sodium salt of betulinic acid was filtered on hot filter and dried at 80oC for 3 hours. White solid product was then added into water (50 ml) and a 1% solution (200 ml) of HCl in water was added dropwise, and stirred for 0.5 hour, filtered, washed with water (2x50 ml) and dried. 14 g of betulinic acid (98% purity) was obtained. The yield of betulinic acid from starting outer birch bark was 0.7%.

Example 10: Betulin separation from birch bark extract

Raw Material	Amount
Birch Bark pellets	100 g
Xylenes	1065 ml
KOH	6.125 g
Water	8.75 ml
Celite 521 (Aldrich)	20 g
Aluminum Oxide (basic)	2.5 g

(A) Extraction

1. Charge a 1 L round-bottomed flask equipped with a magnetic stirring bar, N_2 inlet and teflon covered thermocouple with 500 mL of xylenes, and heat the xylenes to 60°C.
2. Charge 100 g of birch bark pellets, continue stirring and heat to 120°C (+/-) 10°C.
3. Keep the mixture stirred and heated at 120°C (+/-) 10°C for 2 hours.
4. Filter the hot extraction mixture through a 150 mL fine porosity glass frit (pre-washed with hot xylenes). The birch bark was then returned to the

reaction vessel, and was heated with another 450 mL of xylenes at 120°C (+/-) 10°C for ca. 30 min. The batch was filtered through the same filter funnel.

5. The combined xylene extract was re-heated at 100°C(+/-)10°C, and was assayed by HPLC (about 4 drops of xylene solution in 1 ml of MeOH). At this point in the process the HPLC assay showed 3.03A% betulinic acid, 88.87A% betulin, 1.53A% lupeol.

HPLC conditions: LiChroCart-Purosphere Star Rp-8e (5 μ) 4.6 x 150 mm column at 23°C, linear gradient elution of CH_3CN:H_2O from 60:40 to 95:5: in 12 minutes (the water and CH3CN each contain 0.25 mL of TFA per liter), hold 95:5 for 5 minutes, flow 1.0 mL/min, ELSD detection (tube at 100°C, gas flow at 2.2 SLPM, 18 psig). Betulinic acid Rt 8.5 min, betulin Rt 9.0 min, lupeol Rt 16.8 min.

(B) Purification

1. Prepare a solution of 6.125 g of KOH in 8.75 mL of water.
2. Add dropwise the prepared solution of KOH in water to the birch bark extract in xylenes at 90°C (+/-) 10°C with vigorus stirring. Heat to 120°C(+/-10°C).
3. Keep vigorous stirring at 120°C(+/-)10°C and provide azeotropic water distillation (8.75 mL of water should be removed) by refluxing xylenes.
4. About 470 mL of xylene was removed after azeotropic water removal. Do not stop stirring during the process of solvent removal.
5. Filter the hot extraction mixture through a pad of celite 521 (ca. 10 g) on a 60 mL medium porosity glass frit (pre washed with hot xylene).
6. Wash the celite pad with 65 mL of 100°C(+/-10°C) xylene.
7. Keep the KOH treated xylene extract (slightly yellow solution) stirred at 100°C (+/- 10°C).
8. Add 2.5 g of aluminum oxide (Acros catalog # 18999, activated, basic), and continue stirring at 100°C (+/-10°C) for 1 hour.
9. Filter the hot xylene extraction mixture through a pad of celite 521 (ca. 10 g g) on a 60 mL medium porosity glass frit (pre washed with hot xylenes).

10. Wash the celite pad with 10 mL of xylenes at 100°C (+/-10°C).

(C) Isolation

1. Remove ca. 250 mL of xylenes by atmospheric distillation.
2. Cool the batch (about 250 mL) down to 0-5°C and then isolate the product by filtering the solid white precipitate of betulin.
3. Wash the cake with 2 x 20 mL of cold (0-5°C) xylenes.
4. Dry the product for 18 hours in a vacuum oven at 65°C to afford 8.46 g of betulin (8.46% yield).
5. The product assayed at this point by HPLC showed no detectable betulinic acid, 99.64A% betulin, 0.09% lupeol. Assay for floc using the sarcosinate method showed a clear solution.

Example 11: Analytical Testing of Purified Sample

The following was performed by J-Star Research, Inc. (South Plainfield, New Jersey) and Regis Technologies, Inc. (Morton Grove, Illinois). The validation protocol for a High Performance Liquid Chromatography (HPLC) assay for Betulinic Acid impurity in Betulin tests the accuracy, precision, linearity, sensitivity, range and specificity.

To asses the suitability of the method for detection of Betulinic Acid in Betulin, the following conditions were selected for use with the HPLC:

Column:	Merck Purospher STAR RP-8, 250 mm x 4.0 mm, 5 micron
Mobile Phase:	A= 0.02% Trifluoroacetic acid (TFA) in water, B= 0.02% TFA in Acetonitrile (CAN)
Flow Rate:	1 mL/min
Profile:	Gradient (see Table 1 below)
Column Temp:	40°C
Ultra Violet (UV) Detection:	205 nm

Concentration: 50 mg/mL

Injection Volume: 5 µL

Needle Wash: Tetrahydrofuran (THF)

Stop Time: 30 minutes (Post Time = 0 minutes).

Table 1: Pump Gradient Program

Time (minutes)	% Mobile Phase A	% Mobile Phase B
0	30	70
20	5	95
22	5	95
22.1	30	70
30	30	70

The approximate expected retention time for Betulinic Acid is 7.7 minutes.

Specificity is determined by an analysis of the diluent blank sample. Specificity of the instrument is acceptable when there is no interference of the blank sample with quantitation of the target sample.

In order to test the linearity of the relationship between the target sample and concentration, five standards are prepared covering the range of 10-120% of the 50 µg/mL target concentration for Betulinic Acid. The peak area response versus concentration is evaluated by linear regression. The slope, intercept and residual sum of squares are reported to provide a linear correlation of not less than 0.99 (1 being perfectly linear).

The sensitivity samples have target concentrations of 12.5 µg/mL and 5 µg/mL Betulinic Acid. These concentrations correspond to 25% and 10% of the target Betulinic Acid concentration and also to 0.025% and 0.01% impurity in Betulin, respectively. The sensitivity is then evaluated on the detection and recovery (60-140%) of these sample concentrations.

Precision of the system is confirmed by the testing of six repeatable samples. Six samples are prepared consisting of Betulinic Acid at 50 µg/mL in diluent or 100% of the target concentration. An additional six samples of 50

μg/mL Betulinic Acid in Betulin are prepared as spiked samples. The second set of samples represents the 0.1% impurity in Betulin. To attain acceptable precision, the relative standard deviation of the sample sets should not exceed 10%.

The range of the analytical method is simply evaluated on the acceptable degree of linearity, precision and accuracy for the major sample peak. Accuracy is inferred based on acceptable results for specificity, linearity and precision. To confirm sample stability, samples of the target concentration of Betulinic Acid (50 μg/mL) in diluent and spiked Betulinic Acid (50 μg/mL) in Betulin are tested over a 48-hour time period. The response variation should not exceed a 10% relative standard deviation.

All publications, patents, and patent documents are incorporated by reference herein, as though individually incorporated by reference. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.